REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

Claim 42 has been revised to define the invention with additional clarity. More specifically, claim 42 has been revised to make it explicit that the cytolytic peptide is non-covalently attached to the lipid layer. Support for the revision can be found throughout the application (see, for example, at page 6, line 6).

Claims 42-49, 51, 53, 54, 58 and 61 stand rejected under 35 USC 102(e) as allegedly being anticipated by Cullis et al. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

Cullis et al discloses a method of delivering drugs to a target site using inherently unstable particles that disintegrate and become fusogenic in the absence of covalently attached stabilizing molecules, such as PEG, lipopeptides, or polymers. Covalent bonds have to be broken before the stabilizing molecule can be detached, and this results in neighboring particles fusing with each other (i.e., becoming fusogenic), and releasing their contents to thereby cause a therapeutic effect.

In contrast, the claimed invention relates to a method of *detecting* cells involving the use of liposome particles that are highly stable. The particles comprise a cytolytic peptide which is non-covalently attached to the lipid layer. The cytolytic peptide is hydrophobic, and is attached to the lipid layer via hydrophobic interactions between the peptide and the hydrocarbon tails of the lipids. In response to a metabolic signal, the cytolytic peptide forms permeabilizing structures such as pores or channels across the bilayer membrane of an otherwise stable liposome. No bonds need to be broken between the peptide and the bilayer, and the liposome does not

disintegrate following the signal; it remains as a stable functional particle. If the cytolytic peptide were to be covalently attached to the bilayer, then it would be impossible for it to form a pore or channel therethrough, and the signal inside the particle would not be released. As the Examiner will appreciate, the peptides of the present invention are non-covalently attached to the bilayer (and not covalently attached), and, thus, free to move therein so that they can form and self-assemble into the permeabilizing structures without the need to break covalent bonds.

It is also noteworthy that the invention of Cullis et al requires that liposomes be prepared in the presence of a peptide, whereas the present invention can utilize standard stable liposomes, and the peptides are added after their preparation.

In addition to the above, Applicants point out that Cullis et al does not disclose a method of detecting a cell type of interest in a sample but instead discloses a method of delivering a drug. The invention claimed in the subject application is novel over Cullis et al as a totally different method is the subject of the instant claims. The claims are also novel over Cullis et al in view of technical features discussed below.

While the Examiner does not make reference to the first embodiment of Cullis et al, for the sake of completeness Applicants point out that it involves covalently attaching PEG to the polar head group of the lipid layer (PE). Once the PEG is cleaved from the PE, the liposome becomes fusogenic and releases or mixes its contents with other liposomes. Hence, the PEG is not itself a cytolytic peptide, its removal merely causes the liposome to become fusogenic. Furthermore, there is no interaction whatsoever with the lipid layer. Hence, this embodiment of Cullis et al does not provide for the presence of a non-covalently attached cytolytic peptide as required by the present claims, and there is no interaction with the layer to act as or mediate the opening of pores or channels. Therefore, the instant claims are novel over this embodiment.

The second embodiment of Cullis et al, which is specifically referred to by the Examiner, involves attaching a lipopeptide to the polar head group of the lipid. This attachment is by a covalent bond, such as an amide bond, between the lipopeptide and the polar head group of the lipid. This would be clear to one skilled in the art from the disclosure between lines 29-49, paragraph 18. In liposomes, normally, the lipopeptide extends outwardly in to the aqueous environment outside the liposome. However, in some cases, the lipopeptide may extend into the bilayer or even flip to the other side of the bilayer. In both cases, it polarizes and partitions towards the more polar aqueous regions of the bilayer. In either case, the lipopeptide is always attached to the head group covalently. In response to a metabolic signal, for example, a drop in pH, the covalent bonds are broken, and the lipopeptide causes fusion of neighboring liposomes by affecting the lipid bilayer of a neighboring liposome, and not itself. The nature of the covalent attachment of the lipopeptide to the head group of the lipid prevents the lipopeptide from acting on the liposome to which it is attached, and only allows it to contact a neighboring liposome. Hence, this embodiment does not provide for the presence of a non-covalently attached cytolytic peptide, as required in the claimed invention.

The third embodiment of Cullis et al, which is also specifically referred to by the Examiner, involves the use of pH sensitive fusogenic polymers of the general structure $[X\text{-}Y]_n$ where Y is ethylene glycol, and n is 1 to 30. The Examiner alleges this embodiment anticipates the claims. However, Applicants submit that the Examiner is incorrect as the PEG polymer is obviously not a peptide according to the claim, and is ostensibly very similar to the case of the PEG embodiment discussed above (first embodiment). Therefore, the claims as presented are novel over this embodiment.

The fourth embodiment of Cullis et al, which is not referred to by the Examiner, involves using virosomes, i.e. membrane-bound viral envelope fusion proteins, that are also covalently attached to the lipid (lipoproteins and glycolipids). This would be clear to one skilled in the art from the disclosure between lines 16-42, paragraph 32. Hence, the claims as presented are novel over this embodiment for the same reasons that they are novel over the second embodiment.

In view of the above, the Examiner is urged to reconsider his position and withdraw the rejection.

Claim 50 stands rejected under 35 USC 103 as allegedly being obvious over Cullis et al in view of Li et al. The rejection is traversed.

Applicants submit that the particles disclosed in Cullis et al would not work in the claimed method for detecting cells. Applicants have found that the instability and structure of the liposomes or peptide-liposomes modified according to Cullis et al result in non-specific background signal and leakage in the absence of any activating condition. The purpose of the present invention is to target and respond specifically to target cells, which are often present in very low numbers relative to non-target cells. Even low levels of non-specific background become significant, for example, in the detection of, or response to, trace cell populations, such as pathogens and cancer cells. The present invention involves the production of highly stable liposomes, purposefully unsuited to fusion and such instabilities as the particles of Cullis et al, and produces a response by the action of the non-covalently attached cytolytic peptides which are free to form permeabilizing structures or pores in the bilayer membrane of otherwise stable liposomes. If the peptides were covalently attached, they would not be free to form pores or channels in the bilayer, and according to Cullis et al, would need to be cleaved-off. The non-covalently attached cytolytic peptides need to be free to form and self-assemble into such

permeabilizing structures without any covalent attachment, which would otherwise prevent their assembly into permeabilization structures. Hence, the present invention specifically rules out covalent attachment. This is a key feature, which renders the claims novel and unobvious over Cullis et al.

In addition, it would have been totally unexpected in view of Cullis et al, and the technical field in general, that non-covalently attached peptides that are allowed to remain free to form such permeabilizing structures in the presence of conditions wrought at the surface of the target cells, would not also damage the target cells. The target cells, as well as the liposomes themselves, are surrounded by a bilayer membrane. Therefore, it would have been expected from the literature, and reasons for covalent attachment of Cullis et al, that the cytolytic peptide would also permeabilize the target cells and inhibit or prevent the metabolic conditions required to activate the peptides to form the permeabilization structures in liposomes. However, surprisingly, the target cells remain undamaged. Therefore, it was totally unexpected that highly stable liposomes could be used with a non-covalently attached cytolytic peptide, which is free to partition, potentially, into the target cells as well as the liposomes, to selectively permeabilize only the liposomes, and leave the target cells free to metabolize and grow.

Applicants have surprisingly found that, using the claimed invention, it is possible to detect less than ten target cells in a large excess (e.g., million fold) population of non-target cells and produce a signal that is visible to the eye. The present invention is thus very sensitive. This high sensitivity is of significant utility in the detection of trace cell populations, such as may be found in pathogen detection in food, or tissue, or cancer cells in tissue.

Li et al adds nothing that would have cured the fundamental failings of Cullis et al described above.

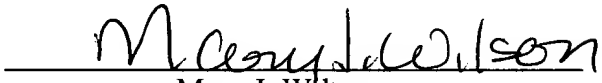
The Examiner is urged to reconsider his position and withdraw the rejection.

Should the Examiner be inclined to maintain either of the rejections after considering the foregoing, he is urged to contact the undersigned by phone (703-816-4011) before issuing an Advisory Action.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 
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